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The hematologic profile of preterm newborns with funisitis

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2013년 7월

서울대학교 대학원
의학과 산부인과학 전공
김은나
The hematologic profile of preterm newborns with funisitis

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Seoul National University
College of Medicine
Eun Na Kim
Introduction

Intrauterine infection or inflammation is found in approximately one third of preterm labor and preterm premature rupture of membranes [1,2]. Certain subsets of preterm fetuses are born with fetal inflammatory syndrome (FIRS), which shares clinicopathologic features with systemic inflammatory response syndrome (SIRS) in adults [3,4]. FIRS is defined by the elevation in fetal plasma IL-6 concentration and multi-organ dysfunction. It is associated with increased incidence of perinatal or long term mortality and morbidity such as bronchopulmonary dysplasia [5-7] and cerebral palsy [8-14]. FIRS features dysfunction of bone marrow [15], heart [16,17], kidney [18], thymus [19,20], skin [21], which are also found in adult SIRS. Consequently, FIRS is associated with the changes in hematologic profiles akin to those found in patients with SIRS [22].

Acute funisitis is a robust inflammation reaction involving umbilical vessels and the Wharton jelly of the umbilical cord. It is a histologic surrogate marker of FIRS [23] and shows intense fetal neutrophilic infiltration into the umbilical vein/artery/Wharton jelly. Fetal plasma IL-6 is significantly elevated in the cases with acute funisitis, more prominently in the presence of umbilical arteritis [24]. However, there is a paucity of information related to the changes in the fetal hematological profiles in the context of acute funisitis. This study was conducted to determine whether the fetal hematological profiles change in preterm neonates with acute funisitis.
Methods

1. Study design

A prospective cohort study was conducted on one hundred and ninety seven consecutive preterm neonates delivered at Seoul National University Hospital. Hematologic profiles of umbilical cord blood at birth was compared between newborns with and without funisitis who met the following criteria: 1) gestational age at birth before 34 completed weeks of gestation; 2) singleton pregnancy; 3) admission due to preterm labor or preterm PROM (premature rupture of membranes); 4) documented placental pathology findings; 5) newborns without major anomaly, Rh isoimmunization or fetal death. The Institutional Review Board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes.

2. Fetal blood

Umbilical cord blood was collected in ethylene diamine tetra-acetic acid containing blood collection tubes by venipuncture of the umbilical vein at the time of delivery. Cord blood was analyzed for WBC count, differential count (neutrophil, monocyte, lymphocyte, basophil, and eosinophil), RBC count, hemoglobin concentration, hematocrit, nucleated RBC and platelet count by XE-2100 automated hematology analyzer (Sysmex America, Inc., Mundelein, Il, U.S.A). The nucleated red cells were determined by morphological evaluation of 100 cells. Since blood cell count varies with gestational age, the observed values were corrected for gestational age by the ratio between the observed and expected mean values for gestational age according to the reference ranges for each gestational age obtained from previous studies.
Eosinophil and basophil counts were not corrected by gestational age because they do not change with gestational age [26]. Leukocytosis, neutrophilia, and monocytosis were defined as > 95th percentile of the same gestational age group, whereas leukopenia, neutropenia, and monocytopenia were defined as < 5th percentile of the same gestational age group. [25]. The result of nucleated red blood cell count was reported as count per 100 WBC.

3. Diagnosis of acute funisitis

Funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton jelly according to the criteria previously published [27].

4. Statistical analysis

The Kolmogorov-Smirnov test was used to determine if the data was normally distributed. The student test was used to compare continuous parametric variables. A two-tailed Mann-Whitney U test was used to compare continuous nonparametric variables. The comparisons of proportions between two groups were performed using chi square or Fisher’s exact tests. A p value of < 0.05 was considered statistically significant. Statistical analysis was performed with SPSS version 18.0 (SPSS Inc. Chicago, USA)
Results

1. Characteristics of the study population

One hundred and ninety seven cases met the inclusion criteria. Funisitis was present in 22% (44/197) of cases. Fifty-three percent (104/197) of mothers were admitted due to preterm labor and 47% (93/197) were admitted due to preterm PROM. Table I compares the clinical characteristics of the study population according to the presence or absence of funisitis. The cases with funisitis had a significantly lower gestational age at birth than those without funisitis (p<0.05).

2. Leukocytosis, neutrophilia, and monocytosis

Figures 1-3 display WBC, neutrophil and monocyte counts according to the presence or absence of funisitis. Newborns with funisitis had significantly higher median total WBC and corrected WBC count, absolute and corrected neutrophil count, and monocyte and corrected monocyte count than those without funisitis (p<0.005 for each). Table II illustrates the proportions of leukocytosis, leukopenia, neutrophilia, neutropenia, monocytosis, and monocytopenia according to the presence or absence of funisitis. Newborns with funisitis had significantly higher rates of neutrophilia and monocytosis than those without funisitis (p<0.05 for each). However, there were no significant differences in the rates of leukocytosis, leukopenia, neutropenia, and monocytopenia between newborns with and without funisitis (p>0.05 for each).
3. Ratio of differential count to total WBC count

There was no significant difference in the count of absolute lymphocyte, corrected lymphocyte, eosinophil, and basophil (Table II). Moreover there was no difference in the proportions of monocyte, eosinophil, and basophil in the leukocyte between the two groups (p>0.1 for each, Table III). However, the proportion of the lymphocyte to the leukocyte was significantly lower and the proportion of neutrophil to the leukocyte was significantly higher in newborns with funisitis than in those without funisitis. (p=0.001; Table II)

4. RBC count, hemoglobin concentration and platelet count

Newborns with funisitis had a significantly lower median RBC count, corrected RBC, hemoglobin concentration, and corrected hemoglobin concentration than those without funisitis (p<0.05 for each, Figure 4, 5). However, there were no significant differences in the median nucleated RBC count, corrected nucleated RBC, and platelet count between newborns with and without funisitis (p>0.05 for each, Table II).
Discussion

1. Principal findings of this study

The principal findings of this study are: 1) the hematologic profiles of the preterm neonates born with funisitis were different from those without funisitis. 2) The preterm neonates with funisitis had significantly higher leukocyte, neutrophil, and monocyte counts. 3) The proportion of neutrophils among leukocytes was increased in preterm neonates with funisitis, while the proportion of lymphocytes was decreased. 3) Funisitis was associated with significantly decreased RBC count and hemoglobin concentration. There was no difference in lymphocyte, eosinophil, basophil, NRBC, and platelet counts.

2. Increased leukocyte, neutrophil, and monocyte counts with funisitis

In acute chorioamnionitis and funisitis preceded by intra-amniotic infection, the concentrations of proinflammatory cytokines are increased in the amniotic fluid and induce amniotrophic chemotaxis of neutrophils [28]. Neutrophils and monocytes are the first line of innate immune defense against infection; neutrophils have peptides that have broad-spectrum antimicrobial properties against bacteria, viruses, and fungi [29,30]. In our study, cord blood leukocyte, neutrophil, and monocyte counts were higher in cases with funisitis, and these findings are consistent with several previous reports [22,31]. Romero et al. have demonstrated that leukocytes and neutrophil counts are increased in FIRS. Carroll et al. have shown that the fetuses with bacteremia have increased leukocyte and neutrophil counts. In these previous studies, no
change was reported regarding the monocyte counts in FIRS and bacteremia; our study further demonstrates the number of monocytes and the proportion of monocytes among leukocyte are significantly increased. This difference may be due to the difference in the timing of fetal blood sampling. Our cord blood sample was obtained at the time of delivery, whereas previous studies used the blood acquired by antenatal cordocentesis. Therefore, our results reflect the sum effects of funisitis from the beginning of funisitis until delivery. Therefore, monocytosis seems to be a feature of FIRS along with leukocytosis and neutrophilia.

In addition to the increase in the aforementioned specific cell counts, funisitis is associated with phenotypic changes in granulocytes and monocytes consistent in the context of activation such as basal intracellular reactive oxygen species production and oxidative bursts [32]

3. Decreased RBC, hematocrit, and hemoglobin concentration in newborns with funisitis

In previous studies, RBC count and hemoglobin concentration were not significantly changed in cordocentesis samples of FIRS [22,31], whereas in our study, RBC count and hematocrit concentration of neonates were lower with funisitis than without funisitis. Our data showed a slight decrease of RBC count and hemoglobin and no decrease in mean cell hemoglobin and mean cell hemoglobin concentration. This is a typical feature of anemia of chronic disease. Therefore, babies with funisitis may suffer from ‘anemia of chronic disease’. Furthermore, decreased hematocrit can result in reduced oxygen-carrying capacity of the blood and thereby initiate a cascade of ischemic-hypoxemic mucosal injury, which can potentially predispose very
low birth weight infants to necrotizing enterocolitis [33].

4. Ratios of neutrophils, monocytes, and lymphocytes to total WBC

Until the third trimester, lymphocyte occupies a dominant proportion of total WBCs. In near-term babies, neutrophil becomes dominant for the preparation of the transition from the sterile space (in utero) to the nonsterile space (ex utero). In case of intra-amniotic inflammation or infection, the fetal immune system changes the proportion of the lymphocyte and neutrophil as if the fetus is exposed to the extrauterine non-sterile environment after delivery. In this study, the total lymphocyte count did not change, yet the ratio of neutrophil and lymphocyte to total WBC shifted as the relative proportion of neutrophil became predominant. This ratio change is also similar to that of chronic systemic inflammation in adults. Leukocytosis in the adult is associated not only with infectious morbidity but also with noninfectious long-term morbidity [34-36]. In adult, leukocytosis is associated with the prevalence of hypertension [37], increased glucose tolerance, low insulin sensitivity [38], a higher prevalence of organ injury after periods of intense stress [39], and major depression [40]. Similarly, the fetus, mounting systemic inflammatory response in response to intra-amniotic infection, may be prone to noninfectious morbidity in adult life. Barker has emphasized the importance of fetal programming and the significance of intrauterine environment in the development of adult diseases [41]. We propose that the changes in the fetal hematologic profiles associated with funisitis would be a novel feature of fetal programming.
Conclusion

The hematologic profiles of preterm newborns with funisitis are characterized by increased white blood cells, neutrophil, and monocyte counts, and decreased RBC count and hemoglobin concentration. The findings underscore the clinicopathologic significance of intra-amniotic infection/inflammation in fetal health.
References


34. Asadollahi K, Beeching NJ, Gill GV. Leukocytosis as a predictor for non-infective mortality and morbidity. QJM 2010;103:285-92


38. Fritsche A, Haring H, Stumvoll M. [White blood cell count as a
predictor of glucose tolerance and insulin sensitivity. The role of inflammation in the pathogenesis of type 2 diabetes mellitus]. Dtsch Med Wochenschr 2004;129:244-8


<table>
<thead>
<tr>
<th></th>
<th>No funisitis (n=153)</th>
<th>Funisitis (n=44)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.00 (23-45)</td>
<td>32.00 (25-43)</td>
<td>0.627</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>31.1 (20.0-33.9)</td>
<td>29.2 (24.1-33.9)</td>
<td>0.039</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>71 (46.4%)</td>
<td>25 (56.8%)</td>
<td>0.223</td>
</tr>
<tr>
<td>Intervention for delivery</td>
<td>15 (9.8%)</td>
<td>2 (4.5%)</td>
<td>0.371†</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>66 (43.1%)</td>
<td>14 (31.8%)</td>
<td>0.178</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1620 (320-3720)</td>
<td>1515 (530-2490)</td>
<td>0.076</td>
</tr>
<tr>
<td>Fetal sex (male)</td>
<td>93 (60.8%)</td>
<td>22 (50.0%)</td>
<td>0.201</td>
</tr>
<tr>
<td>Fetal growth restriction</td>
<td>5 (3.3%)</td>
<td>0 (0.0%)</td>
<td>0.225</td>
</tr>
<tr>
<td>Tocolytics use*</td>
<td>96 (63.6%)</td>
<td>32 (72.7%)</td>
<td>0.261</td>
</tr>
<tr>
<td>Antenatal steroid use*</td>
<td>110 (72.8%)</td>
<td>38 (86.4%)</td>
<td>0.065</td>
</tr>
<tr>
<td>Antibiotics use prior to delivery*</td>
<td>94 (62.3%)</td>
<td>34 (77.3%)</td>
<td>0.065</td>
</tr>
<tr>
<td>Initial event of admission</td>
<td></td>
<td></td>
<td>0.938</td>
</tr>
<tr>
<td>Preterm labor</td>
<td>81 (52.9%)</td>
<td>23 (52.3%)</td>
<td></td>
</tr>
<tr>
<td>Preterm PROM</td>
<td>72 (47.1%)</td>
<td>21 (47.7%)</td>
<td></td>
</tr>
<tr>
<td>Clinical chorioamnionitis</td>
<td>3 (2.5%)</td>
<td>3 (9.1%)§</td>
<td>0.115†</td>
</tr>
<tr>
<td>Fetal distress</td>
<td>24 (15.7%)</td>
<td>3 (6.8%)</td>
<td>0.211†</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>13 (8.5%)</td>
<td>0 (0.0%)</td>
<td>0.077†</td>
</tr>
<tr>
<td>Apgar score 1 min &lt; 7</td>
<td>104 (68.0%)</td>
<td>28 (63.6%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Apgar score 5 min &lt; 7</td>
<td>50 (32.7%)</td>
<td>16 (36.4%)</td>
<td>0.648</td>
</tr>
</tbody>
</table>

Values were expressed as number (percent) or median (range)
GA: Gestational age, *N=151, † Fisher’s exact test, ‡ n=120, § n=33
Table II. Hematologic profile of the fetuses with and without funisitis

<table>
<thead>
<tr>
<th></th>
<th>No funisitis (n=153)</th>
<th>Funisitis (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leukocytosis (%)</strong></td>
<td>94 (61.4%)</td>
<td>34 (77.3%)</td>
<td>0.052</td>
</tr>
<tr>
<td><strong>Leukopenia (%)</strong></td>
<td>3 (2.0%)</td>
<td>1 (2.3%)</td>
<td>1.000$^|$</td>
</tr>
<tr>
<td><strong>Neutrophilia (%)</strong></td>
<td>119 (77.8%)</td>
<td>41 (93.2%)</td>
<td>0.027$^|$</td>
</tr>
<tr>
<td><strong>Neutropenia (%)</strong></td>
<td>6 (3.9%)</td>
<td>0 (0.0%)</td>
<td>0.341$^|$</td>
</tr>
<tr>
<td><strong>Monocytosis (%)</strong></td>
<td>98 (64.1%)</td>
<td>36 (81.8%)</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>Monocytopenia (%)</strong></td>
<td>5 (3.3%)</td>
<td>0 (0.0%)</td>
<td>0.589$^|$</td>
</tr>
<tr>
<td><strong>Lymphocyte (x10^9/L)</strong></td>
<td>3.2 (0.6-15.1)</td>
<td>3.5 (0.8-13.2)</td>
<td>0.713</td>
</tr>
<tr>
<td>Corrected Lymphocyte $^\dagger$</td>
<td>0.8 (0.2-4.0)</td>
<td>1.0 (0.2-4.4)</td>
<td>0.527</td>
</tr>
<tr>
<td><strong>Eosinophil (x10^9/L)</strong></td>
<td>0.1 (0-3.5)</td>
<td>0.1 (0-2.7)</td>
<td>0.85</td>
</tr>
<tr>
<td>Basophil (x10^9/L)</td>
<td>0 (0-1.1)</td>
<td>0 (0-0.4)</td>
<td>0.992</td>
</tr>
<tr>
<td><strong>Nucleated RBC (/100 WBC)</strong></td>
<td>5.0 (0-214)</td>
<td>5.0 (0-149)</td>
<td>0.989</td>
</tr>
<tr>
<td>Corrected Nucleated RBC $^|$</td>
<td>0.3 (0.0-10.2)</td>
<td>0.3 (0.0-10.2)</td>
<td>0.888</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td>44.8 (26-55)</td>
<td>40.8 (29-62)</td>
<td>0.021$^|$</td>
</tr>
<tr>
<td>Corrected Hematocrit $^|$</td>
<td>1.0 (0.7-1.4)</td>
<td>1.0 (0.7-1.4)</td>
<td>0.040$^|$</td>
</tr>
<tr>
<td><strong>Platelet (x10^9/L)</strong></td>
<td>227.0 (10-491)$^*$</td>
<td>258.0 (122-446)$^\dagger$</td>
<td>0.074$^\dagger$</td>
</tr>
<tr>
<td>Corrected Platelet $^|$</td>
<td>1.0 (0.0-2.0)$^*$</td>
<td>1.9 (0.5-1.8)$^\dagger$</td>
<td>0.100$^\dagger$</td>
</tr>
<tr>
<td><strong>MCV (mean corpuscular volume, fL)</strong></td>
<td>115.3 (91.4-151.0)</td>
<td>114.7 (102.0-146.4)</td>
<td>0.644</td>
</tr>
<tr>
<td>Corrected MCV $^\dagger$</td>
<td>1.0 (0.7-1.2)</td>
<td>1.0 (0.8-1.2)</td>
<td>0.498$^\dagger$</td>
</tr>
<tr>
<td><strong>MCH (mean cell hemoglobin)</strong></td>
<td>47.7 (30.7-47.0)</td>
<td>37.7 (33.5-46.0)</td>
<td>0.691$^\dagger$</td>
</tr>
<tr>
<td><strong>MCHC (mean cell hemoglobin concentration)</strong></td>
<td>33.1 (27.4-37.6)</td>
<td>32.9(29.6-35.5)</td>
<td>0.880$^\dagger$</td>
</tr>
</tbody>
</table>

$^\*$n=149, $^\dagger$n=43, $^\dagger$ Student t test, $^\|$ Fisher’s exact test

$^\dagger$ Observed values were corrected for fetal age by calculating ratio between the observed and expected mean value for gestational age
<table>
<thead>
<tr>
<th></th>
<th>No funisitis (n=153)</th>
<th>Funisitis (n= 44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of neutrophil (%)</td>
<td>43.0 (3.0-89.6)</td>
<td>54.4 (5.0- 85.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Percentage of monocyte (%)</td>
<td>7.9 (0-25)</td>
<td>8.0 (2-37)</td>
<td>0.487</td>
</tr>
<tr>
<td>Percentage of lymphocyte (%)</td>
<td>43.9 (6-89)</td>
<td>32.5 (8-76)</td>
<td>0.001</td>
</tr>
<tr>
<td>Percentage of eosinophil (%)</td>
<td>1.8 (0-12)</td>
<td>1.2(0-14)</td>
<td>0.423</td>
</tr>
<tr>
<td>Percentage of basophil (%)</td>
<td>0.0 (0-11)</td>
<td>0.0 (0-3)</td>
<td>0.732</td>
</tr>
</tbody>
</table>
Figure 1. Fetal WBC counts.

(A) Fetuses with funisitis had higher median WBC count than those without funisitis (WBC median 12.6 [range 2.3-35.4] vs. 8.2 [range 2.2-30.4] x 10⁹/L; p= 0.001). (B) Corrected WBC by gestational age was also higher in the fetuses with funisitis. (Corrected median WBC 2.4 [range 0.6-8.2] vs. median 1.5 [range 0.5-7.6]; p=0.001)
Figure 2. Fetal neutrophil counts.

(A) Median absolute neutrophil counts were higher in fetuses with funisitis than in those without funisitis (absolute neutrophil median 7.1 [range 0.5-25.2] vs. 3.6 [range 0.1-23.4] x 10^9/L, p<0.0001) (B) Corrected median absolute neutrophil counts were higher in fetuses with funisitis than in those without funisitis. (Corrected neutrophil count median 13.5 [range1.2-68.9] vs. 4.5 [range 0.2-82.1], p<0.0001).
Figure 3. Fetal monocyte counts.

(A) Median absolute monocyte counts with funisitis were higher than those without funisitis (monocyte median 0.9 [range 0.2-5.8] vs. 0.6 [range 0-3.5] x10^9/L, p=0.003) (B) Corrected monocyte median was higher in fetuses with funisitis than in those without funisitis (corrected monocyte median 4.3 [range 0.8-41.5] vs. 2.7 [range 0-18.3], p=0.001).
Fetuses with funisitis have lower median RBC count than those without funisitis (RBC median 3.6 [range 2.7-5.4] vs. 3.9 [range 2.0-5.5] × 10¹²/L, p=0.012 (A); corrected RBC median 0.98 (range 2.7-5.4) vs. 1.07 (range 2.0-5.5); p=0.023 (B)).
Figure 5. Fetal hemoglobin concentration

Funisitis was associated with lower hemoglobin concentration (hemoglobin median 13.2 [range 9.7-20.6] vs. 14.5 [range 8.1-18.2] g/dL, p=0.005 (A); corrected hemoglobin median 1.0 [range 0.8-1.5] vs. 1.1 [range 0.7-1.4]; p=0.007 (B).
초 록

목적: 조산아에서 태아염증반응증후군(FIRS)의 지표인 태반 제대염이 동반되는 경우 출생 시 혈액 계수가 어떻게 변화하는지 알아보고자 하였다.

방법: 조기진통 및 조기양막파수로 서울대학교 병원에 입원하여 34주 이전에 분만한 산모 197명을 대상으로 하여 태반 제대염의 유무에 따른 태아의 제대혈액 계수의 차이를 알아보았다. 분만 시 제대혈액을 통해 태아의 정맥혈을 채취하여 혈액 계수를 구하였다. 태아 혈액 계수는 주수에 따라 수가 변하기 때문에 측정된 값을 각 주수의 알려진 중앙값으로 나누어 그 비율을 측정하였다.

결과: 1) 태반 제대염의 빈도는 22.3% (44/197) 였다. 2) 태반 제대염이 있을 때 총 백혈구, 중성구, 단핵구 수의 중앙값이 더 높았고 (p<0.01) 중성구 증가증이 더 자주 발생하였다 (p<0.05). 태반에 제대염이 있는 경우 백혈구 내 중성구의 비율이 증가하였고 림프구의 비율은 감소하였다 (p<0.01). 3) 적혈구 수, 혈색소 농도 및 혈마토크랏은 태반 제대염이 있는 경우 유의하게 낮았다 (p<0.05). 4) 그러나 호산구, 호염기구, 유핵적혈구, 혈소판 수는 유의한 변화를 보이지 않았다. (p>0.1)

결론: 태반의 제대염이 있는 조산아의 경우 혈액 내 백혈구, 중성구, 단핵구의 수가 증가하고 적혈구 수와 혈색소 농도가 감소한다.

주요어: 조산, 전신 염증 반응증후군, 태반 제대염, 혈액 계수
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